

Claims

- [c1] 1. A method for continuous amplification of DNA, the method repeating DNA amplification reactions continuously by:
- with a recirculation path and a pump furnished in the recirculation path, feeding unidirectionally through the recirculation path a reaction mixture containing DNA fragments and a reagent solution, held in a reaction-mixture tank;
- sending the reaction mixture within the recirculation path by way of, in order, a denaturing isothermal tank wherein a temperature for dissolving apart the DNA's double strands is maintained, an annealing isothermal tank wherein a temperature at which primers contained in the reagent solution anneal to the DNA fragments is maintained, and an elongation isothermal tank wherein a temperature at which complementary chains are extended continuously onto the primers is maintained; and
- with heat-exchange fluids within the isothermal tanks, maintaining the reaction mixture for predetermined times at predetermined temperatures, and subsequently recirculating the reaction mixture into the reaction-mixture tank.
- [c2] 2. The DNA continuous amplification method set forth in claim 1, wherein the amplification reactions are performed by making time for heat-exchange in the denaturing isothermal tank a reference time, and setting individual heat-exchange times in the annealing isothermal tank and the elongation isothermal tank as multiples of the reference time.
- [c3] 3. A method for continuous amplification of DNA, the method repeating DNA amplification reactions continuously by:
- circuit-feeding unidirectionally through an endless recirculation path, using a pump provided therein, a reaction mixture containing DNA fragments and a reagent solution, held within the recirculation path;
- circulating the reaction mixture within the recirculation path by way of, in order, a denaturing isothermal tank wherein a temperature for dissolving apart the DNA's double strands is maintained, an annealing isothermal tank wherein a temperature at which primers contained in the reagent solution anneal to the DNA fragments is maintained, and an elongation isothermal tank wherein a temperature at which complementary chains are extended continuously onto

the primers is maintained; and
with heat-exchange fluids within the isothermal tanks, maintaining the reaction mixture for predetermined times at predetermined temperatures.

- [c4] 4. An apparatus for continuous amplification of DNA, comprising:
a reaction-mixture tank for holding a reaction mixture containing DNA fragments and a reagent solution;
a denaturing isothermal tank for holding a heat-exchange fluid adjusted to a temperature for dissolving apart the DNA's double strands;
an annealing isothermal tank for holding a heat-exchange fluid adjusted to a temperature at which primers contained in the reagent solution anneal to the DNA fragments;
an elongation isothermal tank for holding a heat-exchange fluid adjusted to a temperature at which complementary chains are extended continuously onto the primers;
a recirculation-path system through which the reaction mixture in the reaction-mixture tank is fed and guided, the recirculation-path system being arranged such that it circuits from the reaction-mixture tank and goes by way of the denaturing isothermal tank, the annealing isothermal tank, and the elongation isothermal tank back to the reaction-mixture tank; and
a pump working to feed the reaction mixture in said recirculation-path system unidirectionally through it; wherein
the apparatus is configured such that the reaction mixture in said recirculation-path system is for timed intervals maintained at prescribed temperatures determined by the heat-exchange fluids in the isothermal tanks.

- [c5] 5. The DNA continuous amplification apparatus set forth in claim 4, wherein said denaturing isothermal tank, said annealing isothermal tank, and said elongation isothermal tank each respectively include:
a container body for holding the heat-exchange fluid;
a heat source for heating the heat-exchange fluid to, and retaining it at, the prescribed temperatures; and
a stirring device for stirring the heat-exchange fluid.

- [c6] 6. The DNA continuous amplification apparatus set forth in claim 4, wherein said denaturing isothermal tank, said annealing isothermal tank, and said elongation isothermal tank each respectively include:
a container body for holding the heat-exchange fluid;
a stirring device for stirring the heat-exchange fluid; and
a heating device containing a pump for circuit-feeding the heat-exchange fluid in between the container body and the heating device, and a heat source for heating the heat-exchange fluid to and retaining it at prescribed temperatures, wherein said heating device supplies the heat-exchange fluid to said container body.
- [c7] 7. The DNA continuous amplification apparatus set forth in claim 4, wherein a plurality of said recirculation-path systems in parallel is provided along with said pump between the reaction-mixture tank and said container bodies.
- [c8] 8. The DNA continuous amplification apparatus set forth in claim 5, wherein a plurality of said recirculation-path systems in parallel is provided along with said pump between the reaction-mixture tank and said container bodies.
- [c9] 9. The DNA continuous amplification apparatus set forth in claim 6, wherein a plurality of said recirculation-path systems in parallel is provided along with said pump between the reaction-mixture tank and said container bodies.
- [c10] 10. The DNA continuous amplification apparatus set forth in claim 4, further comprising:
a plurality of sets of said denaturing isothermal tank, said annealing isothermal tank, and said elongation isothermal tank; and
a plurality of said recirculation-path systems in parallel, provided along with said pump between the reaction-mixture tanks and the isothermal tanks of said plurality of sets.
- [c11] 11. The DNA continuous amplification apparatus set forth in claim 5, further comprising:
a plurality of sets of said denaturing isothermal tank, said annealing isothermal tank, and said elongation isothermal tank; and

a plurality of said recirculation-path systems in parallel, provided along with said pump between the reaction-mixture tanks and the isothermal tanks of said plurality of sets.

- [c12] 12. The DNA continuous amplification apparatus set forth in claim 6, further comprising:
a plurality of sets of said denaturing isothermal tank, said annealing isothermal tank, and said elongation isothermal tank; and
a plurality of said recirculation-path systems in parallel, provided along with said pump between the reaction-mixture tanks and the isothermal tanks of said plurality of sets.
- [c13] 13. The DNA continuous amplification apparatus set forth in claim 4, further comprising a coiled heat-exchange path immersed into each of said isothermal tanks in sections along the way of said recirculation-path system.
- [c14] 14. The DNA continuous amplification apparatus set forth in claim 5, further comprising a coiled heat-exchange path immersed into each of said isothermal tanks in sections along the way of said recirculation-path system.
- [c15] 15. The DNA continuous amplification apparatus set forth in claim 6, further comprising a coiled heat-exchange path immersed into each of said isothermal tanks in sections along the way of said recirculation-path system.
- [c16] 16. The DNA continuous amplification apparatus set forth in claim 7, further comprising a coiled heat-exchange path immersed into each of said isothermal tanks in sections along the way of said recirculation-path system.
- [c17] 17. The DNA continuous amplification apparatus set forth in claim 10, further comprising a coiled heat-exchange path immersed into each of said isothermal tanks in sections along the way of said recirculation-path system.